Xanthomonas Species Causing Bacterial Spot of Tomato in the Russian Federation

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Abstract

Bacterial spot of tomato, caused by *Xanthomonas euvesicatoria* (Group A), *X. vesicatoria* (Group B), *X. perforans* (group C) and *X. gardneri* (Group D) (Jones et al., 2004), formerly known as *X. campestris pv. vesicatoria,* has become very important in the Russian Federation. Leaf spots and wilt symptoms were observed in 2006 in tomato fields located in southern European part of Russia. Field symptoms were first observed in early July of 2006 and had spread to over 30% of the plants in some fields by late August. Yellow-pigmented *Xanthomonas*-like bacteria were isolated from plants using yeast extract-$CaCO_3$ agar. Forty-three original strains were cloned and characterized based on morphologic and biochemical properties, by genetic analysis including rep-PCR, AP-PCR and gene sequencing. Reference strains included XV153 (group A), NCPPB 422^T (Group B), XV 938 (Group C), XV GA2, XV444 (Group D), and 15 strains stored since 1947. Phenotypic and genetic properties of newly isolated and archived Russian strains were similar. Twenty-three strains that were not amylolytic or pectolytic and failed to utilize cis-aconitic acid were identified as *X. gardneri* and 18 strains that were strongly amylolytic and pectolytic were identified as *X. vesicatoria*; neither *X. euvesicatoria* nor *X. perforans* were found.

INTRODUCTION

Bacterial spot of tomato has spread world-wide and can be caused by *Xanthomonas euvesicatoria* (Group A), *X. vesicatoria* (Group B), *X. perforans* (group C), *X. gardneri* (Group D) (Jones et al., 2004), and *X. campestris* pv. *raphani* (White, 1930). The disease causes large yield losses in tomato in the Russian Federation. The pathogens are transmitted mostly by infested seeds. Unfortunately, lack of data on genetic variability among the pathogens in Russia significantly reduced seed testing efficiency.

Leaf spots on tomato were observed in 2005 and 2006 in tomato fields in the South-West of the Russian Federation (Krasnodar, Stavropol, Alania-Osetia) and the Volga region, in glasshouses in Moscow, Tver, Pskov region, Tatarstan. The pathogens were also isolated from commercial seeds reproduced in different regions. Field symptoms were first observed in early July and had spread to over 30% of the plants in some fields by late August. For the first time we evaluated distribution of black spot agents in different regions of Russia.

MATERIALS AND METHODS

Tomato leaves and stems with early symptoms of the disease were collected across the assayed fields. Samples were stored on ice for isolation and in a plant press for archiving. Isolations were made onto YDC (Schaad et al., 2001).

Yellow-pigmented *Xanthomonas*-like bacteria were isolated from the diseased plants, and 43 strains were retained for pathogenicity, biochemical, and genetic tests.

Collections of original strains from weeds and other crop plants were used in the study. The following known cultures were included for comparisons: XV153 (Group A), NCPPB 422T (Group B), XV 938 (Group C), XV GA2, XV444 (Group D) (Jones et al.,

2004) and 15 strains of *X. vesicatoria* isolated in Russia since 1947 (Table 1). Out-group strains included *X. campestris* pv. *campestris*, *X. campestris* pv. *raphani*, and xanthoonads from sunflower and cereals.

Pathogenicity Tests

Bacterial cultures were grown in liquid NBY overnight at 28°C, and adjusted to 1 x 10^6 cfu/ml for inoculation. Seedlings of susceptible tomato cv. 'Dubok' were placed into a lighted dew chamber overnight and atomized carefully with the inoculum.

Genetic Analysis

New and reference original strains were characterized by genetic analysis including REP-, ERIC-, and BOX- PCR, and AP-PCR using primers for *iaaH* gene (F – 5'-TCC GTG ATG GCG ATG CAG-3'; R – 5'-CCA ACG ACC TGT GGT CGG-3') and C-152 (5'-CTG GCG GCT G-3'). Nearly complete (89 to 97%) genes *gyrB* and operon *Xc0006-Xc0007* (genome of *Xcc* ATCC 33913) with total length of 2117 bp were amplified and sequenced. Genetic distances were calculated (Nei and Kumar, 2000), and trees were constructed by MEGA4 program.

RESULTS AND DISCUSSION

All the cultures isolated as suspect *X. vesicatoria* produced leaf spot lesions on tomato cv. "Dubok". Strains identified later as *X. campestris* pv. *raphani* were tested and caused disease on oilseed rape cv. "Coba" and Savoy cabbage cv. "Wirosa", but were avirulent for cauliflower cv. "Miracle". Thus they belonged to race 3 of *X. campestris* pv. *raphani*, identified by Vicente et al. (2006).

Nearly all the strains from tomato plants isolated in Russia were separated by biochemical traits into groups B and D (Table 2). Twenty-three strains that were not amylolytic and pectolytic and failed to utilize cis-aconitic acid were identified as *X. gardneri* and 18 strains that were strongly amylolytic and pectolytic were identified as *X. vesicatoria*; neither *X. euvesicatoria* nor *X. perforans* were found. Genetic analysis by REP-, ERIC-, BOX-PCR, and AP-PCR fingerprinting and by the gene *gyrB* and operon *Xc0006-Xc0007* sequencing confirmed the physiologic grouping of Russian strains with reference strains of *X. vesicatoria* and *X. gardneri*.

Genetic variation among *X. gardneri* strains was significantly lower than among *X. vesicatoria*. All *X. gardneri* strains had conservative BOX and AP-PCR patterns, and only some variation was observed in REP- and ERIC-PCR profiles (data not shown). Several strains isolated from cabbage, sunflower and oats in the Moscow and North Caucasian regions were grouped together with *X. vesicatoria* based on the obtained sequence analysis. Only seven pectolytic strains isolated from tomato plants in 2007 in Alania-Osetia differed from *X. vesicatoria* and *X. gardneri* by utilization of D-galactose and had sequences of the studied genes closest to the type strain of *X. campestris* pv. *raphani* NCPPB1946T from radish.

CONCLUSIONS

Two of four species of former *X. campestris* pv. *vesicatoria* causing black spot disease of tomatoes have been present in Russia since 1947: *X. vesicatoria* (Group B) and *X. gardneri* (Group D). High uniformity of the strains within *X. gardneri* suggests the important role of seed infection in spread of the disease in the field and glasshouse.

Some strains of xanthomonads infecting brassicas, cereals and sunflower in Moscow region and North-Caucasian region have high genetic similarity to *X. vesicatoria*.

Strains of race 3 *X. campestris (vesicatoria)* pv. *raphani* are causing disease on tomato plants in the region of North Caucasus (Alania-Osetia).

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Literature Cited

Jones, J.B., Lacy, G.H., Bouzar, H., Stall, R.E. and Schaad, N.W. 2004. Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. Syst. Appl. Microbiol. 27(6):755-62.

Nei, M. and Kumar, S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Schaad, N.W., Jones, J.B. and Lacy, G. 2001. *Xanthomonas*. In: N.W. Schaad, J.B. Jones and W. Chun (eds.), Laboratory guide for identification of plant pathogenic bacteria, 3rd edition. APS Press, St. Paul, MN.

da Silva, A.C.R. et al. 2002. Comparison of the genomes of two Xanthomonas pathogens with differing host specificities. Nature 220:459-463.

Vicente, J.G., Everett, B. and Roberts, S. 2006. Identification of Isolates that Cause a Leaf Spot Disease of Brassicas as *Xanthomonas campestris* pv. *raphani* and Pathogenic and Genetic Comparison with Related Pathovars. Phytopathology 96(7):735-745.

Westcott, C. and Horst, R.K. 2001. Westcott's Plant Disease Handbook Springer.

White, H.E. 1930. Bacterial spot of radish and turnip. Phytopathology 20:653-662.

Tables

Table 1. Archived and new strains from Russian Federation.

Strain #	Host	Region	Year
322, 324, 346, 403, 410	Tomato	Voronezh	1948
411, 412	Tomato	Saratov	1948
435	-/-	Voronezh	1947
444	-/-	Stalingrad (Volgograd)	1949
503, 511, 512	-/-	Moscow	1949
5001-7	-/-	Alania (N. Osetia)	2004
Xv1-23	-/-	Tatarstan, Tver	2006
Xv24-41	Tomato	Krasnodar, Pskov	2006

Table 2. Basic physiological properties of xanthomonads isolated from tomato plants in Russia.

Test/ strains	Xv153	403, 410, 503, 56, 198, 415, 417, 432, 938, 5235, Xv1-23	Xv938	332, 324, 346, 411, 412, 435, 444, 511, 512, 153, 197, 991pep, GA2, Xv24-41
	Group A	Group B	Group C	Group D
Amilolytic activity	-	+	+	–
Pectolytic activity	-	+	+	–
Acontinic activity	-	+	+	-
Utilization of : Dextrin	+	+	+	–
Cis-acontic acid	+	–	+	–
D-galactose	+	-	+	-
cis-aconitic acid	+	+	+	-